Pretreating Anaerobic *Listeria monocytogenes* with Propionate Enhances Subsequent Intracellular Infection

Laura McFarland, Chantal Diallo, and Yvonne Sun
University of Dayton, Department of Biology

Background

- ***Listeria monocytogenes*** is a Gram-positive, intracellular pathogen responsible for the foodborne illness Listeriosis.
- **Macrophages** are professional phagocytes and are one of the main immune cells *L. monocytogenes* will encounter.
- **Propionate** is a short chain fatty acid used as an antimicrobial food preservative and produced by our gut microbes. Propionate is known to have anti-inflammatory and immunomodulatory properties.

Main Research Questions

- How does exposure to propionate for various length of times influence the ability of *L. monocytogenes* to enter and replicate inside macrophages?
- How does the presence of oxygen impact the effect of propionate on *L. monocytogenes* infection and intracellular growth?

Research Methods

- **Intracellular CFU:** Gentamicin protection assays were performed with RAW264.7 macrophages infected at an MOI of 10 with *L. monocytogenes* strain 10403s grown overnight aerobically or anaerobically, +/- 25 mM propionate. No propionate cultures were back-diluted +/- propionate for 2 hours. Infected cells were lysed at 2 and 6 hpi; lysates were plated on LB agar to enumerate intracellular CFU.
- **Plaque Assay:** A gentamicin protection assay was performed with confluent L2 fibroblast cells infected with *L. monocytogenes* grown as described above. Plaque diameters were measured three days post infection.

Results

Overnight propionate treatment exhibited no effect on initial *L. monocytogenes* entry and survival at 2 hpi

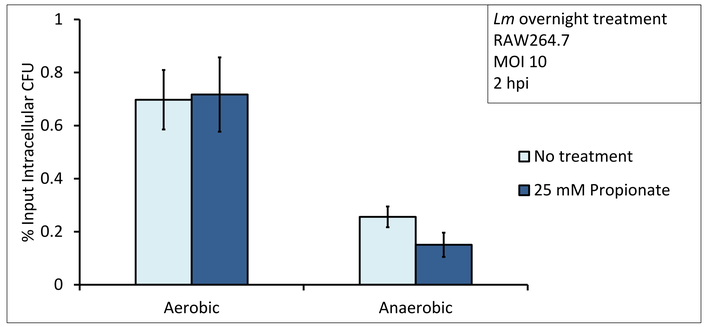

Figure 1. Percent of input *L. monocytogenes* CFU present in RAW 264.7 cell lysates 2 hpi.

Overnight propionate treatment significantly enhanced intracellular growth of anaerobic *L. monocytogenes* between 2 and 6 hpi

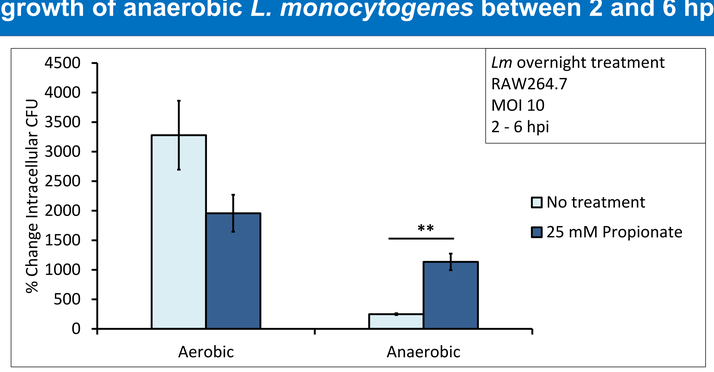

Figure 2. Percent change in *L. monocytogenes* intracellular CFU between 2 and 6 hpi.

2-hour propionate treatment exhibited no effect on initial *L. monocytogenes* entry and survival at 2 hpi

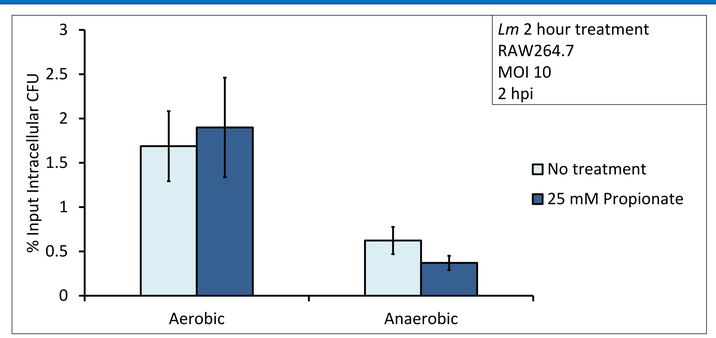

Figure 3. Percent of input *L. monocytogenes* CFU present in RAW 264.7 cell lysates 2 hpi.

Results

2-hour propionate treatment significantly enhanced intracellular growth of anaerobic *L. monocytogenes* between 2 and 6 hpi

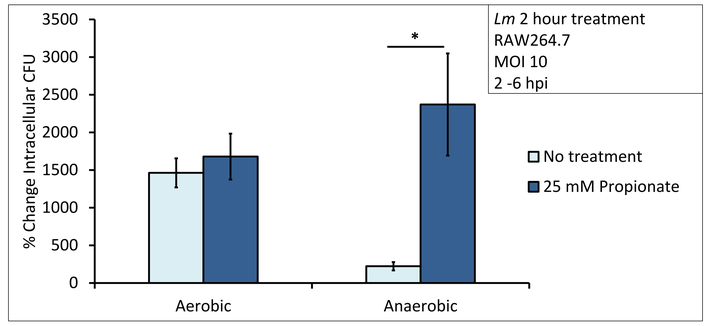

Figure 4. Percent change in *L. monocytogenes* intracellular CFU between 2 and 6 hpi.

Overnight propionate treatment significantly enhanced cell-to-cell spread of anaerobic *L. monocytogenes*

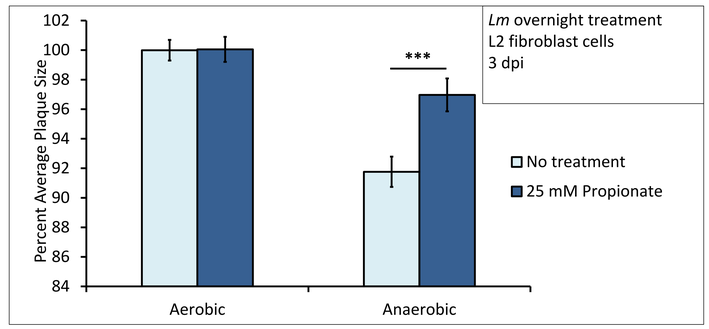

Figure 5. Percent average plaque size 3 days post infection. Aerobic No treatment is set as control at 100%.

Conclusions

- Propionate pretreatment has no significant impact on initial entry and survival of *Listeria monocytogenes* grown aerobically or anaerobically.
- Petreatment of anaerobically, but not aerobically grown *Listeria monocytogenes* causes significantly enhanced subsequent intracellular infection between 2 and 6 hours post infection. Even a short treatment (2 hours) enhances subsequent intracellular infection.
- Pretreatment of anaerobically grown *Listeria monocytogenes* significantly enhances cell-to-cell spread, continuing to affect infection long after the propionate is removed.

University of Dayton

Contact

Laura McFarland
Email: mcfarlandl2@udayton.edu

Chantal Diallo
Email: diallocha10@gmail.com

Yvonne Sun
Email: ysun02@udayton.edu

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